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Two new species of Schizorhynchia (Kalyptorhynchia, Rhabdocoela, Platyhelminthes) from Japan

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Abstract

Schizorhynchia is a group of free-living interstitial flatworms within Kalyptorhynchia, characterized by possessing a split proboscis. To date, only seven species of Schizorhynchia have been recorded from Japan. Here, we describe two new species of schizorhynchs, *Proschizorhynchella quadricaudata* **sp. nov.** and *Cheliplana izuensis* **sp. nov.**, based on specimens collected from the intertidal zone of Japanese waters. We also explored the phylogenetic position of the new taxa based on partial sequences of the nuclear 18S rRNA and 28S rRNA genes. *Proschizorhynchella quadricaudata* **sp. nov.** is characterized by (*i*) proboscis lips with a row of glands; (*ii*) a simple cone-shaped stylet; and (*iii*) a caudal end with four finger-shaped projections. *Cheliplana izuensis* **sp. nov.** can be distinguished from its congeners by (*i*) two strong hooks with bifurcate tips, encircling the distal part of the spiny cirrus; (*ii*) the fact that the proximal 20% of the cirrus length is unarmed; (*iii*) proboscis' sidepieces lacking needles; (*iv*) paired seminal vesicles; and (*v*) the presence of a vagina. The phylogenetic analyses support the position of *P. quadricaudata* **sp. nov.** within Schizorhynchidae and the position of *C. izuensis* **sp. nov.** in a clade containing all species of *Cheliplana* within Cheliplanidae.

Key Words

Cheliplanidae, interstitial, meiofauna, Pacific Ocean, Schizorhynchidae, Turbellaria

Introduction

Schizorhynchia is a group of free-living interstitial flatworms within Kalyptorhynchia with worldwide distribution (Tessens et al. 2014; Diez et al. 2019; Smith III et al. 2020). To date, 192 species of Schizorhynchia have been described (Diez et al. 2019; Gobert et al. 2020; Armonies 2023; Velásquez-Rodriguez et al. 2023; WoRMS 2024). Schizorhynchs are characterized by possessing a split proboscis (a pair of finger-like and dorsoventrally opposed tongues) (Smith III et al. 2015), except for species of *Typhlorhynchus* Laidlaw, 1902, which lack a proboscis. Subsequently, schizorhynchs can be subdivided into two groups by the presence or absence of proboscis hooks (not constituting taxonomic categories). The former belong to Cheliplanidae Schilke, 1970; Diascorhynchidae Meixner, 1928; Karkinorhynchidae Meixner, 1928; and Nematorhynchidae Schilke, 1969; and the latter to

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Schizorhynchidae Graff, 1905 (Tessens et al. 2014; Smith et al. 2015). In Schizorhynchidae, species of *Carcharodorhynchus* Meixner, 1938, and *Serratorhynchus* Noldt, 1988, and a few species of *Thylacorhynchus* de Beauchamp, 1927, have a proboscis armed with denticles (Smith et al. 2015).

The Japanese microturbellarian fauna and rhabdocoels in general are poorly known, and only seven schizorhynch species have been recorded in the country, including *Freddius tricaudatus* Takeda & Kajihara, 2018, *Proschizorhynchella pacifica* (Evdonin, 1969) Noldt, 1985, *P. caudociliata* Takeda & Kajihara, 2018, *P. magnoliae* Takeda & Kajihara, 2018, *P. shibazakii* Takeda & Kajihara, 2018, and *P. shuttlecock* Takeda & Kajihara, 2018. *Cheliplana setosa* Evdonin, 1971 was also recorded to Japan by Ax (2008); however, Gobert et al. (2021) considered that the specimens described by Ax probably belong to *C. hawaiiensis* Gobert, Reygel, Van Steenkiste & Artois, 2021 or *C. evdonini* Karling, 1983. Therefore, it is not currently possible to establish the identity of the species of *Cheliplana* recorded from Japan.

Recently, Van Steenkiste et al. (2023) recorded the eighth Japanese schizorhynch *Cheliplana terminalis* Brunet, 1968. In the latter paper, an undescribed species of *Carcharodorhynchus* Meixner, 1938, the eukalyptorhynch *Parautelga* sp., and the dalytyphloplanid *Trigonostomum vanmecheleni* Artois, Schockaert, Beenaerts & Reygel, 2013 were also recorded from Japan. Seven other marine or brackish water species of Rhabdocoela are documented from Japan: *Palladia nigrescens* (Evdonin, 1971) Evdonin, 1977, *Phonorhynchoides japonicus* Ax, 2008, *Utelga monodon* Ax, 2008, *Pogaina japonica* Ax, 2008, *P. scypha* Ax, 2008, *Promesostoma teshirogii* Ax, 1992, and *Ptychopera japonica* Ax, 2008 (see Ax 1992, 2008).

During faunal surveys on interstitial marine flatworms in Japan, eight specimens belonging to two species of Schizorhynchia were collected, of which we herein describe two new species. Furthermore, we estimated their phylogenetic relationships based on partial sequences of the nuclear 18S rRNA and 28S rRNA genes.

Material and methods

The specimens examined in the present study were collected from intertidal sand at five localities in Japan (Fig. 1): Yoichi (fine-grained sand, lower intertidal; 43°12'00.1"N, 140°46'55.4"E) on 15 May 2019, Manazuru (coarse sand with rocks, lower intertidal, 35°09'42.9"N, 139°08'25.0"E) on 29 October 2019 and 19 March 2021, Imaihama (fine-grained sand, lower intertidal, 34°45'01.0"N, 139°00'15.3"E) on 30 October 2019, Mihama (fine-grained sand, lower intertidal, 34°58'25.6"N, 138°45'50.9"E) on 29 October 2019, and Ishibu (fine-grained sand, lower intertidal, 34°43'58.8"N, 138°45'21.7"E) on 29 October 2019. Sediment samples were agitated with tap water to extract meiobenthic animals. The suspended water was filtered with a 62-µm mesh net. The residue was subsequently transferred into seawater and sorted under a dissecting microscope. Live

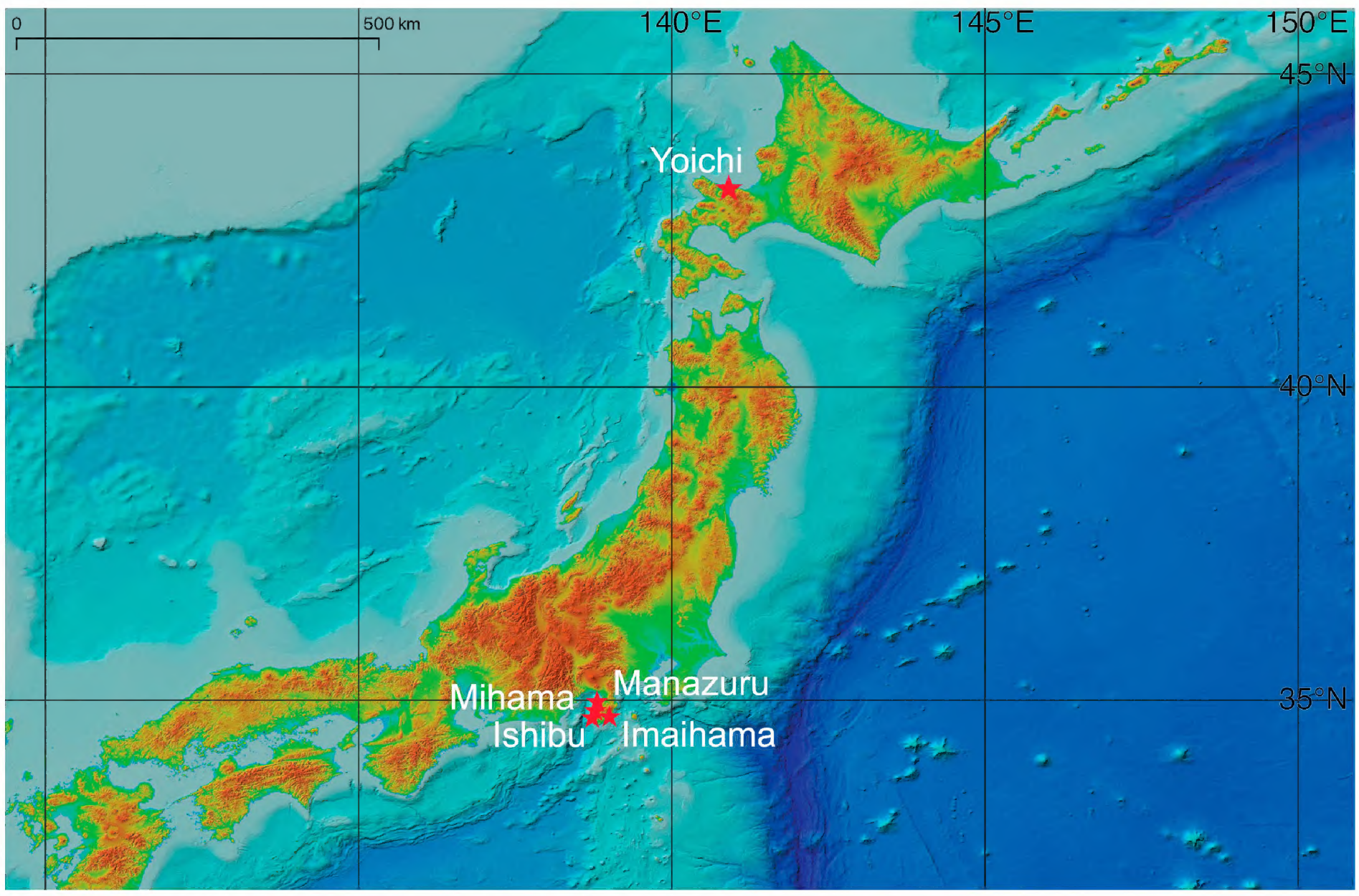

Figure 1. Map of Japan indicating the sampled localities.

specimens were studied, squeezed with seawater, and later fixed in 10% buffered formaldehyde. The holotypes were mounted in glycerol and subsequently sealed with nail polish around the edges of the coverslip. Fresh specimens were photographed using an Olympus E-5 digital SLR camera, observed under Nikon SMZ1500 and Olympus BX51 stereomicroscopes, and photographed using a Nikon D5200 digital camera. The drawings were made based on photographs of fresh specimens using the application Celsys CLIP STUDIO and a pen tablet, Wacom Intuos. Type specimens of the two new species were deposited in the National Museum of Nature and Science, Tsukuba (**NSMT**), Japan.

For morphological observations by scanning electron microscopy (**SEM**), specimens fixed in formaldehyde were washed in deionized water, dehydrated in a graded ethanol series, dried in a Hitachi HCP-2 critical-point dryer using liquid CO_2, coated with gold in a Hitachi E-1045 ion sputter, and observed using a Hitachi S-3000N scanning electron microscope at an acceleration voltage of 15–30 kV.

For the purposes of phylogenetic analyses, total DNA was extracted using a DNeasy Tissue Kit (Qiagen) for one specimen of each species. A reaction mixture [0.2 µl TaKaRa Ex Taq (Takara, Japan), 2 µl of 10× Ex Taq Buffer (Takara, Japan), 1.8 µl dNTP mixture (Takara, Japan), 1 µl of each primer pair (10 µM), 1 µl of extracted DNA, and 14 µl of distilled water] was used for PCR amplification. PCR amplification was performed in an Applied Systems 2720 thermal cycler using the primer pairs 1F/9R (Giribet et al. 1996) for 18S and LSU5/rd5b (Littlewood 1994; Schwendinger and Giribet 2005) for 28S and the following protocol: preheating at 94 °C for 2 min; 35 cycles of 94 °C for 40 s, 52 °C for 75 s, and 72 °C for 60 s; and a final extension at 72 °C for 7 min. Nucleotide sequencing was performed using internal primers in addition to the aforementioned primer pairs with an ABI BigDye Terminator ver. 3.1 Cycle Sequencing Kit and an ABI 3100 Avant Genetic Analyzer (Applied Biosystems). The internal primers used in the present study were as follows: 3F/5R (Giribet et al. 1996) and 18Sbi/S2.0 (Whiting et al. 1997) for 18S, and LSU3/D2F (Littlewood 1994), 28Z (Hillis and Dixon 1991), and Sa (Whiting et al. 1997) for 28S. Newly obtained sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank) under the accession numbers listed in Table 1.

A total of 60 sequences (38 for 18S, 22 for 28S; representing 43 species) were used for molecular phylogenetic analyses. Three sequences were obtained from specimens collected for this study, and the remaining 57 sequences were downloaded from GenBank. All sequences were aligned with MAFFT ver. 7.205 using the E-INS-i strategy (Katoh and Standley 2013). Ambiguous positions were removed using trimAL with the gappyout method (Capella-Gutiérrez et al. 2009). The trimmed alignments of the two genes 18S (1558 bp) and 28S (502 bp) were concatenated using Kakusan program (Tanabe 2007), which recommended a GTR+G evolutionary model for each of the genes. A phylogenetic tree was constructed using the maximum likelihood (ML) method in the RAxML-VI-HPC program (Stamatakis 2006). The robustness of the ML tree was evaluated by 1,000 bootstrap pseudo-replicates (F option). Bayesian Inference (BI) analysis was conducted using Mr. Bayes 3.2.2 (Ronquist et al. 2012), with Markov chains of 10 million generations. Model choice for each partition was also based on the Kakusan

Table 1. List of species included in the phylogenetic analyses, together with the respective GenBank accession numbers. Species names are referred to by Rundell and Leander (2014) and Smith et al. (2015).

Species	18S	28S
Cicerina tetradactyla	KJ887465	KJ887520
Paracicerina laboeica	KJ887408	JN852888
Carcharodorhynchus sp. 1 JPS-2015	KR339017	–
Carcharodorhynchus flavidus	KJ887457	KJ887563
Carcharodorhynchus sp. 2 JPS-2015	KR339018	KR339047
Carcharodorhynchus sp. 3 JPS-2015	KR339019	–
Carcharodorhynchus sp. TJ-2014	KJ887472	–
Carcharodorhynchus sp. 4 JPS-2015	KR339020	–
Carcharodorhynchus sp. 1 NVS-2023	OR490851	OR490867
Carcharodorhynchus sp. 2 NVS-2023	OR490850	OR490866
Carolinorhynchus follybeachensis	KR339021	KR339048
Cheliplana sp. 1 JPS-2015	KR339022	KR339049
***Cheliplana izuensis* sp. nov.**	–	**LC512875**
Cheliplana sp. 2 JPS-2015	KR339023	–
Cheliplana sp. 3 JPS-2015	KR339024	–
Cheliplanilla caudata	KJ887449	KJ887502
Cheliplanilla sp. 1 JPS-2015	KR339025	–
***Proschizorhynchella quadricaudata* sp. nov.**	**LC512873**	**LC512874**
Diascorhynchus rubrus	AJ012508	–
Diascorhynchus serpens	KJ887439	–
Karkinorhynchus bruneti	AY775740	–
Lehardyia alleithoros	KR339032	JF340473
Linguabana tulai	JN205121	–
Proschizorhynchella sp. 1 JPS-2015	KR339042	–
Proschizorhynchus sp. 2 JPS-2015	KR339036	KR339054
Proschizorhynchus sp. 3 JPS-2015	KR339037	KR339055
Proschizorhynchus gullmarensis	–	KJ887532
Proschizorhynchus tricingulatus	KJ887423	KJ887503
Proschizorhynchus trductibus	AY775744	–
Proschizorhynchus sp. 5 JPS-2015	KR339039	–
Proschizorhynchus sp. 6 JPS-2015	KR339040	KR339056
Schizorhynchidae sp. 2 JPS-2015	KR339041	AY340463
Schizorhynchidae sp. 3 JPS-2015	KR339043	–
Schizochilus caecus	AY775745	–
Schizochilus chorius	AY775746	–
Schizochilus sp. 1 JPS-2015	KR339044	KR339059
Schizochilus marcusi	AY775747	–
Schizorhinos vancouverensis	JN205120	–
Schizorhyncoides canaliculatus	AY775748	–
Thylacorhynchus ambronensis	AY775749	–
Thylacorhynchus sp. 1 JPS-2015	KR339045	KR339060
Thylacorhynchus conglobatus	KJ887448	KJ887534
Thylacorhynchus sp. 2 JPS-2015	KR339046	–
Thylacorhynchus sp.	–	KJ887508
Undicola toefinoensis	JN205119	–

results. Run convergence was analyzed using Tracer v1.6 (Rambaut et al. 2018); we assessed convergence by examining the trace plots for each parameter to ensure stationarity and proper mixing and confirmed that the effective sample sizes (ESS) for all parameters were above 200, indicating adequate sampling from the posterior distributions. The first one million generations were discarded as burn-in. Two species of Eukalyptorhynchia, *Cicerina tetradactyla* Giard, 1904, and *Paracicerina laboeica* Meixner, 1928, were used as outgroup taxa.

Results

Systematic account

Platyhelminthes Claus, 1887
Rhabditophora Ehlers, 1985
Rhabdocoela Ehrenberg, 1831
Kalyptorhynchia Graff, 1905
Schizorhynchia Meixner, 1928
Schizorhynchidae Graff, 1905
***Proschizorhynchella* Schilke, 1970**

***Proschizorhynchella quadricaudata* sp. nov.**

https://zoobank.org/29B3324A-5D36-4B51-93E0-A0792BB3BAE0

Figs 2–4

(New Japanese name: Yotsuo-kuchibashi-mushi)

Video material. Video of male copulatory organ of *Proschizorynchella quadricaudata* (https://doi.org/10.6084/m9.figshare.25568148.v1).

Type material. ***Holotype*** • (NSMT-Pl 6475): adult, intertidal sands, Imaihama Beach, 30 Nov. 2019, collected by NJ and NH; • one reference specimen (NSMT-Pl 6476): intertidal sands, Ishibu Beach, 30 Oct. 2019, collected by NJ and NH; • one reference specimen (NSMT-Pl 6477): intertidal sands, Manazuru, 29 Oct. 2019, collected by NJ and NH; • one reference specimen (NSMT-Pl 6478): intertidal sands, Yoichi, 15 May 2019, collected by NJ and NH; • additional specimen: intertidal sands, Mihama, 29 Oct. 2019, collected by NJ and NH; • additional specimen: intertidal sands, Imaihama Beach, 30 Nov. 2019, collected by NJ and NH, used for DNA extraction.

Description. Body 2.2 mm long (1.7–2.3 mm, n = 4), 0.4 mm at its widest point (0.4–0.5 mm, n = 4). Live specimens yellowish and translucent (Fig. 2). Two pairs of short bristles (Figs 3A, 4A: br) and two pairs of small globular papillae (Figs 3A, 4A: gp) at anterior end. Proboscis consisting of two elongated lips, 360 µm in length, 100 µm at the widest part (Figs 2A, B, 3A–C, 4A: pr); a row of glands with 27–37 apertures in the four studied specimens on inner side of proboscis lips (Fig. 3A, B, C: indicated by white arrow, Fig. 4A); gland apertures rectangular; glands measuring 14–16 µm long and 6–8 µm in width in the four studied specimens. Pair of black eyes situated posterior to the proboscis (Figs 2, 4A). Pharynx located at midbody, 160–240 µm in length and 200–260 µm in width in the four studied specimens (Figs 2B, 3G).

One adhesive girdle located at the level of the pharynx, composed of six adhesive papillae arranged at regular intervals (Fig. 3D: indicated by black arrows). Tail with four finger-like projections presents at the posterior end (Figs 3H, 4B: fp), each projection similar in size, 40–50 µm in length and 20–30 µm in width in the four studied specimens, muscles strongly developed at their middle part (Fig. 3H: mc).

Pair of testes, each 180 µm in diameter (Figs 2, 4A), located anterior to the pharynx. Seminal ducts widen and form a pair of seminal vesicles posterior to the pharynx (Figs 3E, 4A, B: sv), each 160 µm in length (150–170 µm, n = 4) and 40 µm in width (40–60 µm, n = 4). Seminal ducts (Fig. 4B: sd) fuse in a single duct just before entering the male copulatory bulb. Male copulatory organ comprising a proximal prostate vesicle and a distal stylet. Prostate vesicle globular, 80 µm both in length and width (60–120 µm, n = 4) (Figs 3E, 4B), separated in two packages; necks of the extracapsular prostate glands enter the copulatory bulb proximally. Stylet enclosed by the male genital duct, cone-shaped, 100–150 µm in length and 20–30 µm in width in the four studied specimens (Fig. 4B: st, mgd). Male genital duct opens into the common atrium (Fig. 3E, F: ca), and the latter leads to the exterior through the common genital pore. Common atrium brownish pigmented due to the secretions of atrial glands.

Female genital system consisting of a vitellarium (Fig. 2A: v) extending between the proboscis and the posterior end of the body, a single ovary (Figs 3E, 4B: ov), and bursa located behind the common genital atrium.

Diagnosis. Species of *Proschizorhynchella* with its tail end exhibiting four finger-like projections. Proboscis with a well-developed row of glands on each lip. Cone-shaped stylet ~100 µm long.

Etymology. The specific name is derived from a Latin prefix "*quadri*" + adjective "*caudata*", referring to the tail with four finger-like projections of the new species.

Distribution and habitat. Northern Japan: Yoichi, on fine-grained sand, lower intertidal. Central Japan: Manazuru, on coarse sand with rocks, lower intertidal; Imaihama (type locality), on fine-grained sand, lower intertidal; Mihama, on fine-grained sand, lower intertidal; and Ishibu, fine-grained sand, lower intertidal.

Remarks. The studied specimens show characteristics that, provisionally, allow us to include them within *Proschizorhynchella*. Species of *Proschizorhynchella* are schizorhynchids characterized by a simple male copulatory bulb forming a spiny cirrus and/or one or two stylets. The stylets are simple funnel structures, spiralized plates, or bear ridges (Schilke 1970; Takeda and Kajihara 2018). The diagnostic characteristics provided by Schilke (1970) are shared by a group of species that likely belong to different genera (Noldt 1985; Karling 1989). As noted by Noldt (1985), the only character differentiating species of *Proschizorhynchella* from species of *Proschizorhynchus* Meixner, 1928, is the lack of the curved stylet in the

Figure 2. *Proschizorhynchella quadricaudata* sp. nov., live specimen. **A.** Holotype (NSMT-Pl 6475); **B.** Reference specimen (NSMT-Pl 6478). **A.** Whole specimen, dorsal view, pharynx discharged; **B.** Whole specimen, latero-dorsal view. Scale bars: 1 mm (**A**); 0.3 mm (**B**). Abbreviations: b, bursa; c, male copulatory organ; ca, common atrium; g, glands; oss, orange spherical structure; mgd, male genital duct; ov, ovary; ph, pharynx; s, stylet; sv, seminal vesicle; t, testis; v, vitellarium.

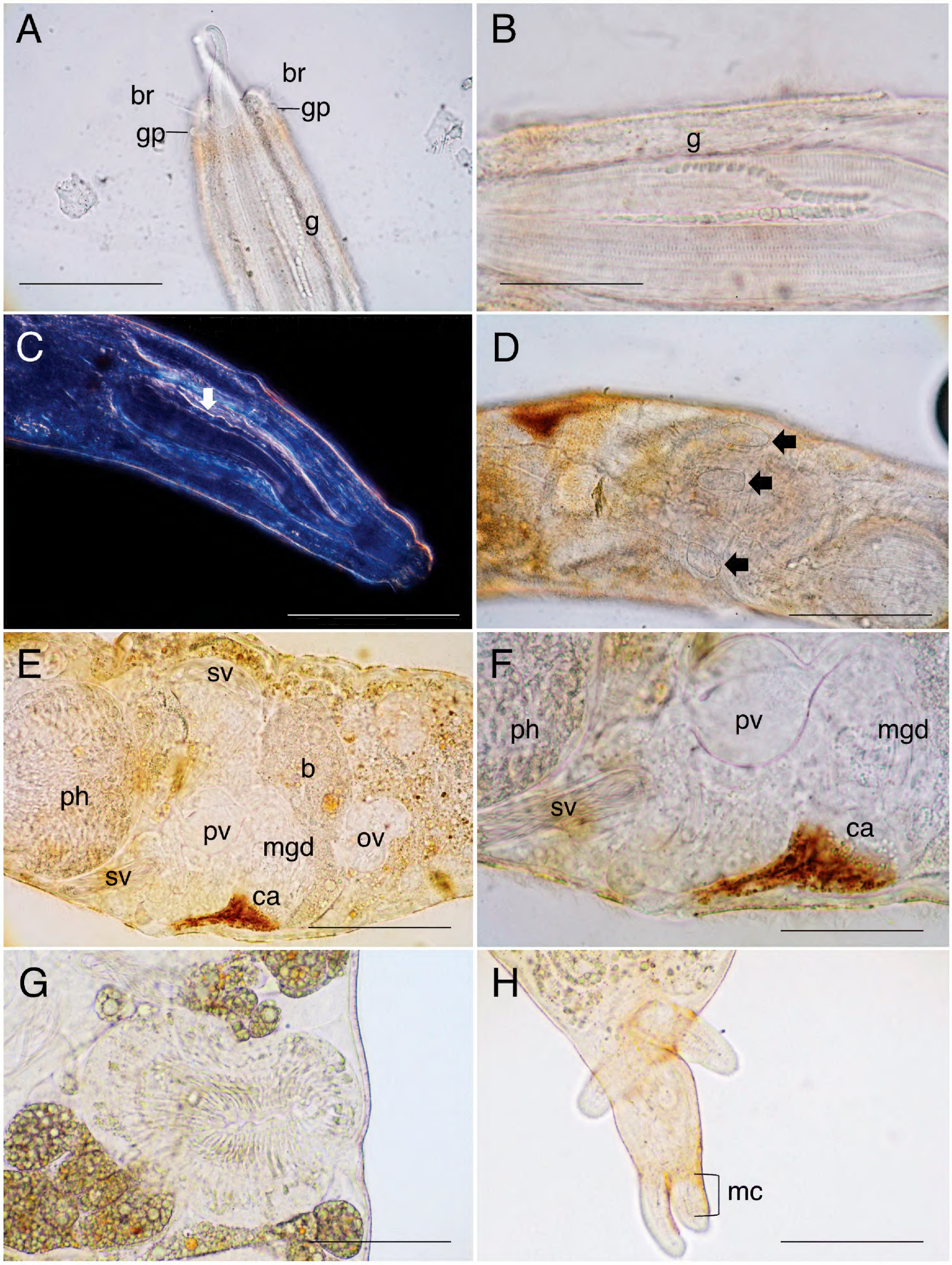

Figure 3. *Proschizorhynchella quadricaudata* sp. nov., live specimen, (**A–F**) reference specimen (NSMT-Pl 6478), (**G, H**) holotype (NSMT-Pl 6475). **A.** Anterior end; **B.** Proboscis with glands row; **C.** DIC view of proboscis with glands row (indicated by arrow); **D.** Midbody area; adhesive papillae are indicated by dashed lines (indicated by arrow); **E.** Overview of reproductive organs; **F.** Copulatory organ and common atrium; **G.** Pharynx; **H.** Tail with four finger-like projections. Scale bars: 200 µm (**A**); 100 µm (**B**); 200 µm (**C–E**); 100 µm (**F–H**). Abbreviations: br, bristles; ca, common atrium; g, glands; gp, globular papillae; mc, muscles; mgd, male genital duct; ov, ovary; ph, pharynx; pv, prostate vesicle; s, stylet; sv, seminal vesicle; t, testis.

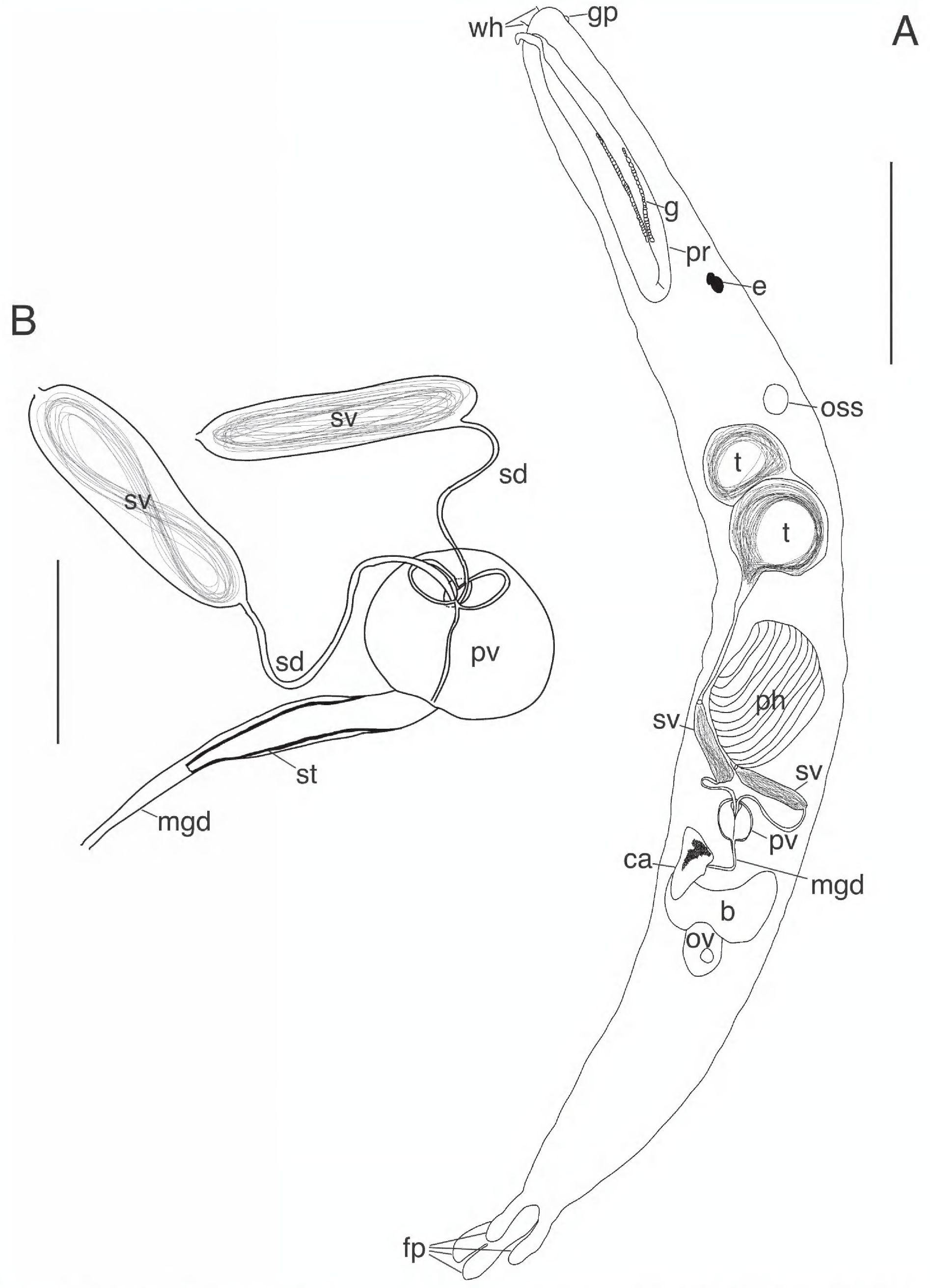

Figure 4. *Proschizorhynchella quadricaudata* sp. nov., drawing based on a reference specimen (NSMT-Pl 6478). **A.** Whole body; **B.** Male copulatory organ with stylet. Scale bars: 300 µm (**A**); 100 µm (**B**). Abbreviations: b, bursa; c, cirrus; ca, common atrium; e, eye; g, glands; gb, globular papillae; mgd, male genital duct; oss, orange spherical structure; ov, ovary; ph, pharynx; pr, proboscis; pv, prostate vesicle; sd, seminal duct; st, stylet; sv, seminal vesicle; t, testis; v, vitellarium; w, whisker.

former. However, the presence of a sclerotized mouthpiece in the bursa of species of *Proschizorhynchus* easily allows their differentiation from species of *Proschizorhynchella* (Armonies 2023).

Karling (1989) elaborated more on the idea that *Proschizorhynchella* includes a paraphyletic group of species and questioned the use of the pharynx position and testes number to differentiate among schizorhynchid genera. However, these authors and posterior ones (Takeda and Kajihara 2018) did not refine the diagnosis of *Proschizorhynchella* or make other taxonomical changes. Unfortunately, the morphological disparities among species of *Proschizorhynchella* have not been tested through molecular phylogenetic analyses, and none of the 18 previously known species of the genus has been molecularly tested (see Discussion). Therefore, we refrain from updating the diagnosis of *Proschizorhynchella* until more molecular and morphological data are available to elaborate a comprehensive reclassification of this taxon.

Among the other 18 known species of *Proschizorhynchella*, *P. quadricaudata* sp. nov. can easily be differentiated because of the four finger-like projections of its posterior end, a characteristic not present in its congeners. The row of glands that opens along its proboscis lips is also unique in this species, and this character has only been reported for an undescribed species of *Proschizorhynchella* (see Smith III et al. 2015: Fig. 3A).

Other characteristics are very variable among species of *Proschizorhynchella* (see Takeda and Kajihara 2018). Most species have two eyes, two testes, and one gonopore. However, the number of adhesive belts varies from 0 to 3. *Proschizorhynchella quadricaudata* sp. nov. exhibits a single adhesive belt at the level of the pharynx. Only three other species of *Proschizorhynchella* present a single belt: *P. bivaginata* Schilke, 1970; *P. robusta* Noldt, 1989; and *P. schilkei* Karling, 1989. *Proschizorhynchella bivaginata* is unique among these species because it has a cirrus in the copulatory bulb, whereas the other species have a stylet. *Proschizorhynchella schilkei* has two pairs of testes, and its stylet is a curved, comma-shaped hook, while *P. robusta* has a spiralized stylet (Karling 1989; Noldt 1989). In contrast, the stylet of *P. quadricaudata* sp. nov. is a simple and more or less straight tube.

Cheliplanidae Schilke, 1970

Cheliplana izuensis sp. nov.

https://zoobank.org/9DC8EDB8-D1D7-4F49-8885-EDE52FE571FE

Figs 5–7

(New Japanese name: Izu-kuchibashi-mushi)

Video material. Video of male copulatory organ of *Cheliplana izuensis* (https://doi.org/10.6084/m9.figshare.25568256.v1).

Type material. ***Holotype*** • (NSMT-Pl 6479): intertidal sands, Imaihama Beach, 30 Oct. 2019 collected by NJ. Two reference specimens were collected together with the holotype, one used for SEM observation (NSMT-Pl 6480) and the other for DNA extraction.

Description. Body 2 mm long and 0.38 mm at its widest point. Live animals brownish and translucent (Fig. 5A), without eyes. Proboscis 100 µm long and 50 µm at its widest point (Fig. 5A, B: pr), with one pair of hooks and one pair of sidepieces (Figs 6A, 7A: h & sp, respectively). Proboscis hooks 15 µm long, 3 µm wide at the base, smooth and curved. Sidepieces straight, cylindrical in shape, not bifurcated. Pharynx 350 µm long and 150 µm wide (Figs 5A, B, 6A). Prepharyngeal cavity with spines (Fig. 6A: cp). One adhesive ring with duo-adhesive glands, without papillae located in the subcaudal body end (Fig. 6A: dashed line indicated by black arrow Fig. 7B: ar).

One testis located anterolaterally to the pharynx, 690 µm long and 70 µm wide (Figs 5A, 6A: t). Atrial organs and ovary located in the caudal body fourth. Seminal ducts form a pair of seminal vesicles at the beginning of the caudal body fourth, each 100 µm long and 20 µm wide (Fig. 6A, B: sv). Seminal vesicles fuse in a single duct just before entering the male copulatory bulb. Male copulatory bulb comprising a prostate vesicle and a cirrus. Prostate vesicle pear-shaped, 100 µm long and 65 µm wide. Male duct forms in its distal half a cirrus, which separates by a sphincter (Fig. 5D: sph, Fig. 6B: ed, sp). Cirrus 65 µm long, 20 µm wide, consisting of three sections (Fig. 6B): (*i*) proximal unarmed region (approximately proximal 20% of its length), (*ii*) armed part with fine spines (approximately 15 µm long), and (*iii*) rhomboid area at distal end of cirrus, armed with fine spines (approximately 20 µm long) and two strong hooks (22 µm long) with bifurcate tips (encircling most distal part of cirrus).

Vitellarium extending from posterior region of pharynx to the caudal body end (Figs 5A, 6A: v). Ovary located posterior to the male copulatory bulb. Common gonopore located just caudally to the male copulatory bulb. Vagina present, a ring of circular muscles surrounding its aperture to the outside.

Etymology. The specific name is derived from a Latin adjective referring to the type locality in the Izu Peninsula.

Distribution and habitat. The new species is only known from the type locality (Imaihama, Izu Peninsula, Shizuoka), fine-grained sand in the lower intertidal.

Remarks. At present, the genus *Cheliplana* contains 49 species (Gobert et al. 2021). *Cheliplana izuensis* sp. nov. is placed in the genus *Cheliplana* due to having a pair of curved proboscis hooks, soft proboscis sidepieces, a single adhesive girdle, and a cylindrical, barrel-shaped pharynx with a long pre-pharyngeal cavity (de Beauchamp 1927; Schilke 1970; Gobert et al. 2017, 2021). It is distinguished from its congeners by the following combination of characteristics: (*i*) two strong hooks with bifurcate tips, encircling the distal part of the cirrus; (*ii*) a cirrus with the proximal 20% of its length unarmed, posteriorly armed with 15-µm-long and fine spines; (*iii*) proboscis sidepieces lacking bristles; (*iv*) paired seminal vesicles; and (*v*) the presence of a vagina.

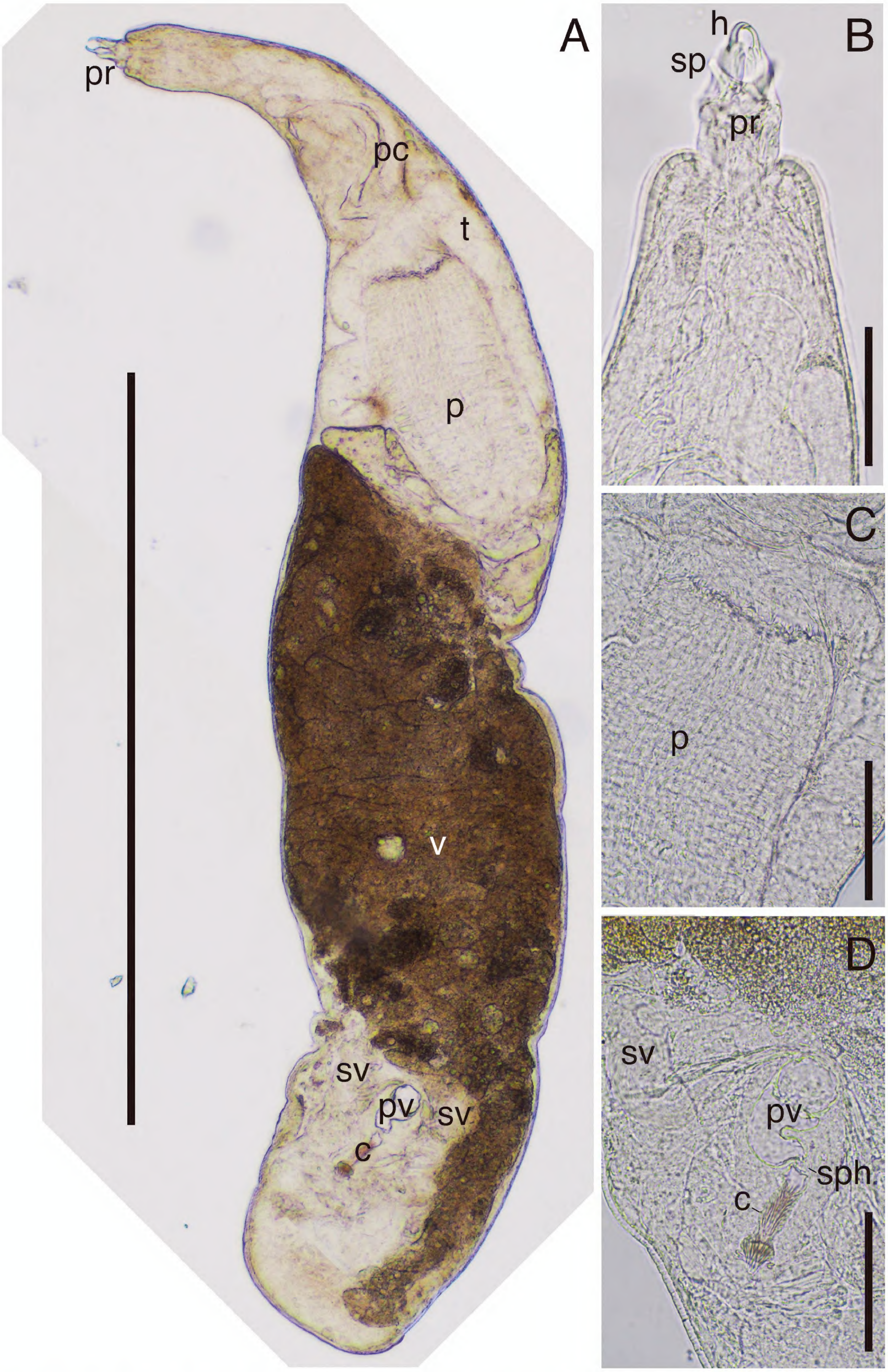

Figure 5. *Cheliplana izuensis* sp. nov., live specimen, holotype (NSMT-Pl 6479). **A.** Whole specimen, dorsal view; **B.** Anterior end; **C.** Pharynx; **D.** Overview of internal organs. Scale bars: 1 mm (**A**); 100 µm (**B–D**). Abbreviations: c, cirrus; h, hook; pr, proboscis; pc, proboscis cavity; t, testis; p, pharynx; pv, prostate vesicle; sp, side piece; sph, sphincter; sv, seminal vesicle; v, vitellarium.

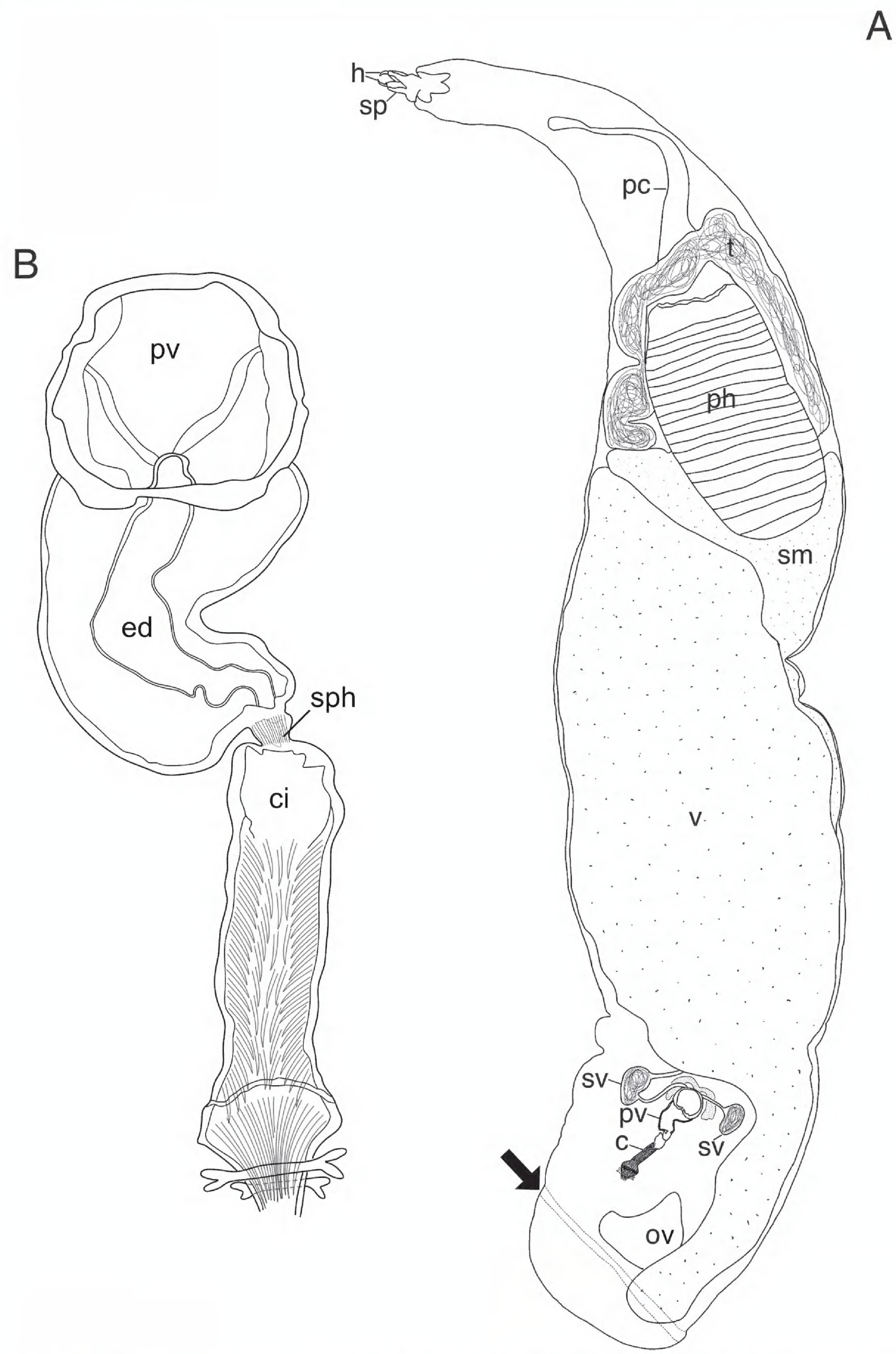

Figure 6. *Cheliplana izuensis* sp. nov., drawing based on holotype (NSMT-Pl 6479). **A.** Whole body; **B.** Male prostate vesicle and cirrus. Scale bars: 5 mm (**A**); 100 µm (**B**). Abbreviations: c, cirrus; cp, cuticular pockets; ed, ejaculatory duct; h, hook; pr, proboscis; pc, proboscis cavity; pv, prostate vesicle; t, testis; p, pharynx; sp, side piece; sm, stomach; sph, sphincter; sv, seminal vesicle; ov, ovary; v, vitellarium. Break line indicates adhesive girdle.

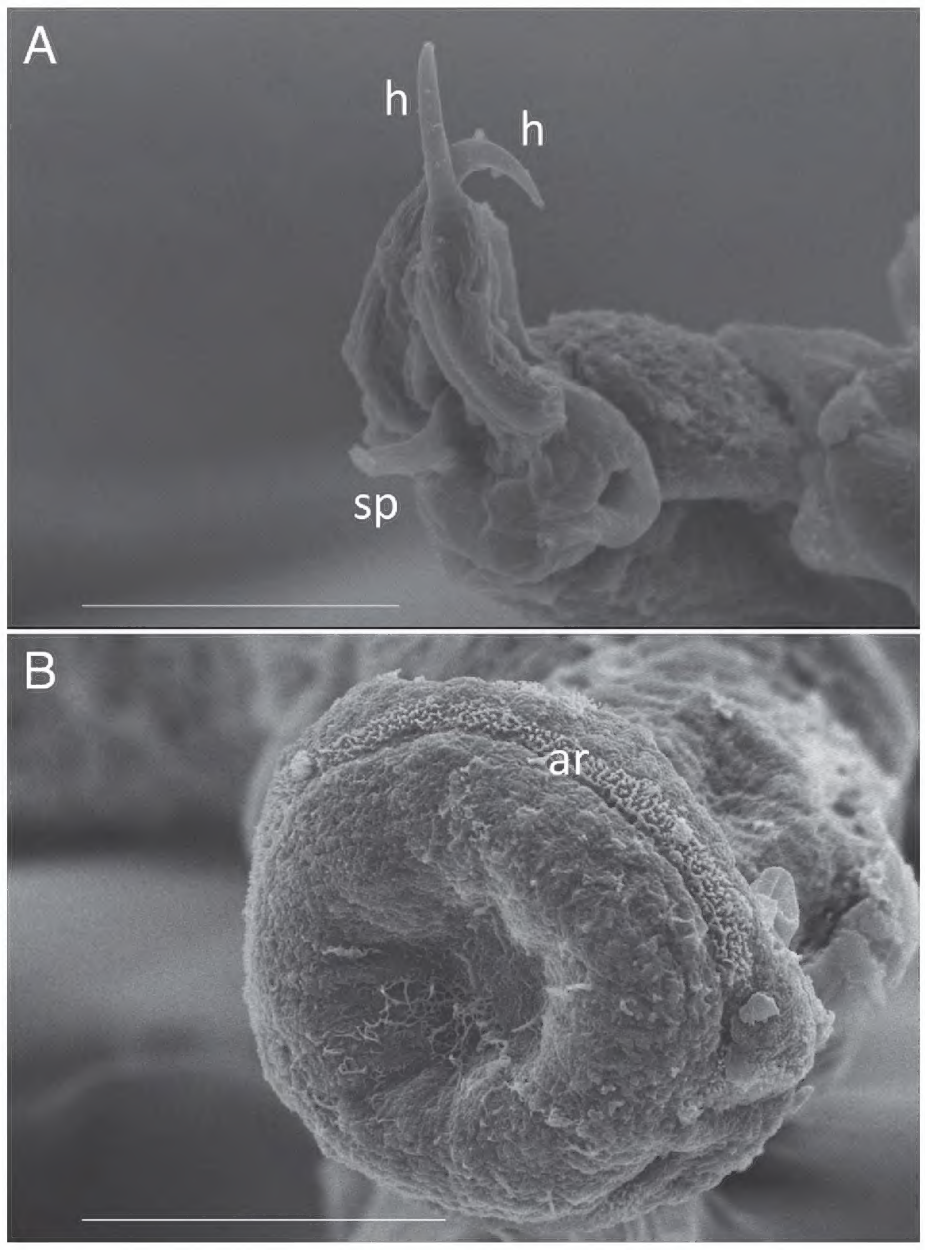

Figure 7. SEM observation of *Cheliplana izuensis* sp. nov., reference specimen (NSMT-Pl 6480). **A.** Proboscis; **B.** Posterior end. Scale bars: 30 µm (**A**); 50 µm (**B**). Abbreviations: ar, adhesive ring. Arrows indicate hooks. Arrowheads indicate sidepieces.

The morphology of the cirrus of *C. izuensis* sp. nov. allows us to state that the new species is related to *C. evdonini* Karling, 1983, and *C. setosa* Evdonin, 1971. This morphological similarity was tested using the identification key provided by Gobert et al. (2021), and, indeed, the three species have a convergent morphology of the cirrus. Therefore, we will mainly compare the new species with the other two morphologically similar ones. The cirrus in these three species has differentiated regions (unarmed or armed with spines differing in size) and includes hooks (*C. evdonini* and *C. izuensis* sp. nov.) or well-differentiated spines (*C. setosa*) more or less organized in a ring. In *C. izuensis* sp. nov., two hooks occur subterminally in the cirrus, whereas six hooks occur about the midlength of the cirrus in *C. evdonini* (Karling 1983), and 5–6 strong spines are present at the beginning of the distal third of the cirrus in *C. setosa* (Gobert et al. 2021). The hooks are distally pointing in *C. evdonini* and *C. setosa* but rounded and bifurcate at both ends in *C. izuensis* sp. nov. Furthermore, the proximal unarmed part of the cirrus distinguishes *C. izuensis* sp. nov. from the other two related species that have the cirrus armed of spines over its whole length. Another possible related species, *C. pusilla* Brunet, 1968, shows a multi-part cirrus; however, it was considered to belong to a different morphological species group due to the lack of hooks in the cirrus as occurs in the previously mentioned species (Gobert et al. 2021). Moreover, *C. izuensis* sp. nov. is differentiable from *C. pusilla* because in the latter species the cirrus shows two distinct spiny regions, a proximal curved area armed with very fine spines and a distal asymmetric one displaying larger spines (Brunet 1968).

The number and morphology of the seminal vesicles are also important to differentiate species of *Cheliplana* (see Diez et al. 2019; Gobert et al. 2021). *Cheliplana izuensis* sp. nov. presents two seminal vesicles, as is the case in *C. setosa*, whereas there is only one in *C. evdonini*. However, it was not clearly observed if both seminal vesicles are connected to the testis in *C. izuensis* sp. nov., while one of the vesicles is blind in *C. setosa*. Nevertheless, both seminal vesicles contain sperm in *C. izuensis* sp. nov., and, therefore, we can suppose that, indeed, they are connected to the testis. Among these three species, *C. izuensis* sp. nov. is also unique by the fact that the proboscis sidepieces do not bear bristles.

Phylogenetic analyses

The phylogenetic analysis, based on concatenated sequences of 18S and 28S rDNA genes, is convergent with those previously performed by Tessens et al. (2014) and Smith III et al. (2015) and evidences the poorly known and defined relationships within Schizorhynchia. Both the Bayesian and ML topologies were congruent (Fig. 8). *Proschizorhynchella quadricaudata* sp. nov. is included in a poorly supported clade including most species of Schizorhynchidae, Diascorhynchidae, and Karkinorhynchidae (pp = 0.98; BS = 52). *Cheliplana izuensis* sp. nov. is included in a clade containing all species of *Cheliplana* (pp = 0.98; BS = 51).

Discussion

Considering the results of our phylogenetic analysis, it is not possible to address the phylogenetic relationships of the taxa within Schizorhynchidae, except for the well-supported clade, including species of *Schizochilus* Boaden, 1963. Tessens et al. (2014) also found this limitation, suggesting that more species of Schizorhynchidae need to be included in molecular phylogenetic analyses (they remain underrepresented) and/or other, maybe more informative, molecular markers need to be used. Indeed, we did not find support for the monophyly of *Proschizorhynchella* due to *P. quadricaudata* sp. nov. not forming a clade with *Proschizorhynchella* sp. We expected that these two species would form a clade because of shared morphological characteristics, such as the belt of glands in the proboscis lips. Considering the morphological variability among species of *Proschizorhynchella* (see a detailed comparison in Takeda and Kajihara 2018), we can suspect that the genus is not monophyletic (see previous remarks on *P. quadricaudata* sp. nov.). On the other hand, species of *Cheliplana*, including *C. izuensis* sp. nov., form a monophyletic group, which is sister to species of *Cheliplanilla*. Therefore, we were able to resolve the relationships between these two genera, a question elusive in previous studies (Smith III et al. 2015).

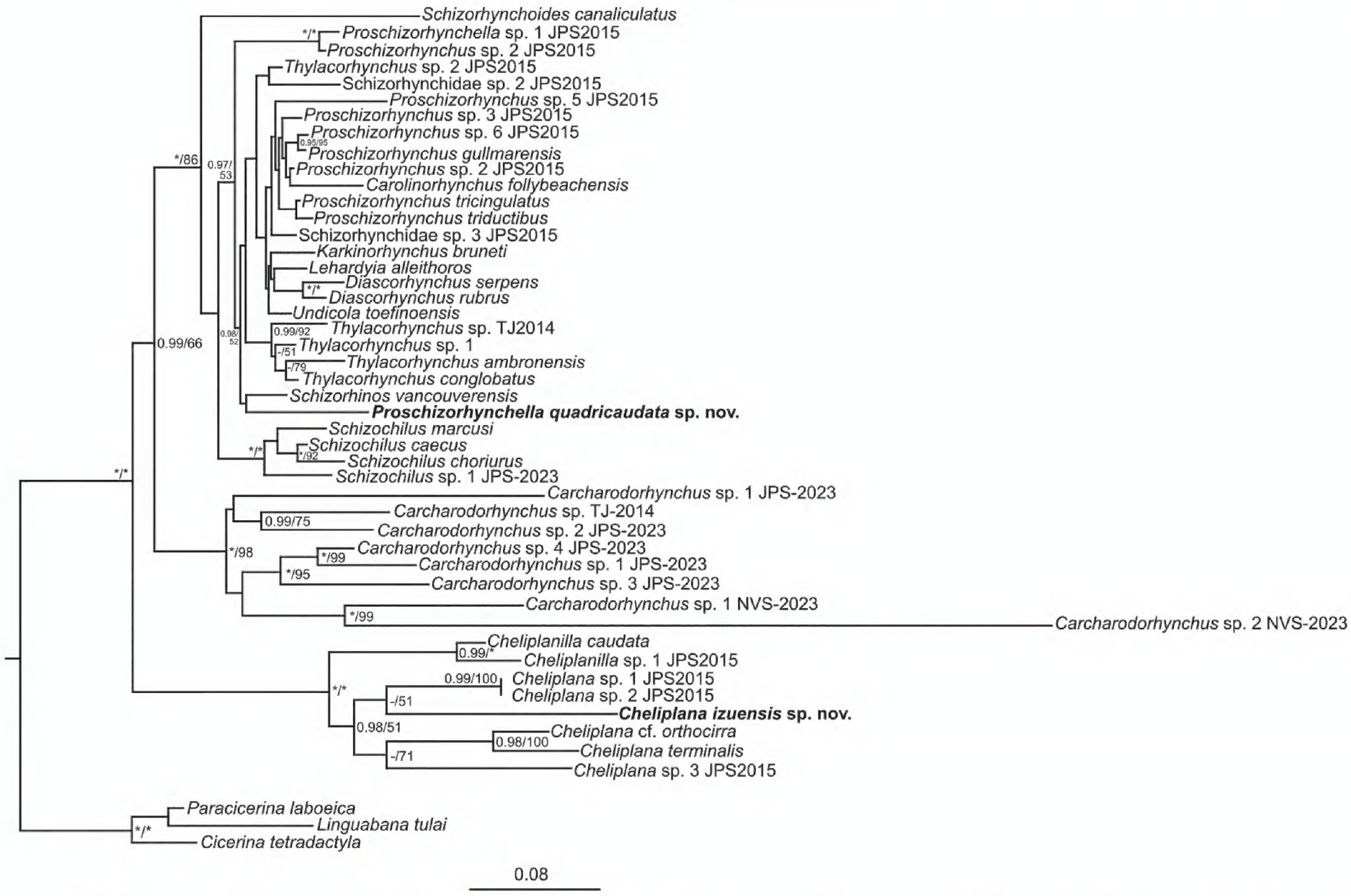

Figure 8. Maximum-likelihood phylogenetic tree of Schizorhynchia based on concatenated 18S and 28S rDNA sequences. The posterior probability of each branch is shown, followed by the bootstrapping values. More than 50% are indicated for each branch.

As of now, nine of the 20 rhabdocoel species recorded from Japan (see Tsuyuki et al. 2024), including the two new species here described, belong to Schizorhynchia. We suspect that the richness of this taxon in this region is higher, as in other recently studied archipelagos (Gobert et al. 2017; Diez et al. 2019, 2023). Most probably, the species richness of its sister taxon 'Eukalyptorhynchia' and microturbellarians as a whole is as high as in other areas of the world. While the macroturbellarian Polycladida have been well documented from Japan (e.g., Oya et al. 2022; Tsuyuki et al. 2022, 2023), major efforts will be necessary in the future to sample and conduct broad taxonomical studies on microturbellarians in this region. Understanding the diversity of free-living flatworms, particularly microturbellarians, is crucial to understanding relevant ecosystem processes. These little animals play important roles as predators and biomass producers, and their study is relevant not only for taxonomical purposes but also to address species and ecosystem conservation.

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